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Original Article

Biocompatibility and cytotoxic effects of myofunctional appliance materials on human periodontal ligament fibroblasts

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Abstract *Background/purpose:* Prefabricated myofunctional devices are widely used in children's dentistry and early orthodontics. This study investigated the effects of materials used in myofunctional appliances on the viability of human periodontal ligament fibroblasts (HPLFs), inflammatory responses, and bone remodeling under simulated oral conditions. The focus was on biocompatibility and cytotoxicity to ensure safety in clinical applications.
Materials and methods: Four materials—EF Line (EF), ProOrtho (PO), Myobrace (MB), and Invisalign (IV)—were tested under conditions with and without artificial saliva (AS). HPLFs were cultured and exposed to eluates from these materials for 24, 48, and 72 h. Cell viability was measured using the MTT assay, and protein expression of inflammatory and bone remodeling markers (COX-2, IL-1, IL-6, TNF-α, ALP, OPG, RANKL) was evaluated using Western blotting.
Results: A 30 % AS concentration had minimal impact on cell viability and was used in subsequent experiments. EF showed significant cytotoxicity and elevated inflammatory protein expression, particularly IL-6 and COX-2, peaking at 48 and 72 h. PO exhibited moderate effects, while IV and MB maintained higher cell viability and lower inflammatory responses, similar to the control group. For bone remodeling markers, EF demonstrated high RANKL expression and low ALP/OPG levels, indicating bone resorption potential. In contrast, IV and MB had minimal impact on bone remodeling, maintaining a favorable RANKL/OPG ratio.
Conclusion: MB and IV demonstrated higher biocompatibility, minimal inflammatory effects,

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and stable bone remodeling properties. EF and PO exhibited higher cytotoxicity and inflammatory potential, maybe needed further material modifications to improve properties.

Introduction

The concept of Oral Myofunctional Therapy (OMT) was introduced as early as 1939 by Rogers,[1] who proposed that proper oral muscular function should be established through exercises, rather than assuming it would naturally follow the establishment of good occlusion. He emphasized that OMT is not a "panacea for all orthodontic problems."[2] Over time, OMT has evolved and is now defined as "the treatment of dysfunctions of the muscles of the face and mouth, with the purpose of correcting orofacial functions, such as chewing and swallowing, and promoting nasal breathing."

Despite its advancements, the efficacy of OMT remains controversial in orthodontic literature. Critics argue that incorrect tongue function, infant bottle feeding, and improper leaning and sleeping habits are the primary causes of malocclusion. However, Straub WJ supported Rogers' theory and further elaborated on the role of incorrect tongue function and improper oral habits in malocclusion.[3,4]

On the other hand, Profit moved away from Straub's emphasis on tongue thrust during swallowing and instead highlighted the significance of resting tongue position and nasal respiration as critical factors in orthodontic outcomes.[5] These differing perspectives illustrate the ongoing debate about the role of OMT in addressing orofacial dysfunctions and its influence on malocclusion.

Prefabricated myofunctional devices are widely used in pediatric dentistry and early orthodontic treatments. The fundamental concept is to support normal breathing while improving the development of the maxilla and mandible and achieving better alignment of teeth. These devices also aim to correct oropharyngeal muscle dysfunction, thereby enhancing oral health, physical appearance, tongue posture, and airway volume.

Common materials for early Orthodontic Myofunctional Therapy (OMT) devices include soft elastomeric materials, primarily composed of polyurethane, polyvinyl chloride (PVC), or similar compounds. For aligners used in orthodontics, materials such as polyethylene terephthalate-co-1,4-cyclohexylenedimethylene terephthalate (PETG) and thermoplastic polyurethanes (TPU) are frequently utilized. These materials are chosen for their flexibility, durability, and compatibility with oral environments.

Due to the prolonged wearing of OMT devices and their constant contact with oral mucosa or gingival tissues, the biological safety of these devices is crucial. The oral environment may influence the release of harmful substances or metal ions from the devices, raising safety concerns.

Studies have investigated the safety of devices such as Myobrace (MB) and LM Trainer™ 2 (LMD). In vitro tests on human keratinocytes showed that cell viability dropped to 82 % at pH 3 with LMD exposure. Anti-apoptotic markers (Bcl-2 and Bcl-xL) showed slight increases, while the pro-apoptotic marker (Bad) decreased. No significant capillary toxicity was observed, with an irritation score below 0.9.[6] According to the international standard ISO 10993—5:2009 (Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity), a survival rate above 70 % is regarded as non-cytotoxic, whereas values below 70 % are considered indicative of cytotoxic potential.

One review article discusses the effectiveness and biocompatibility of different orthodontic aligner materials, evaluating five commonly used types: polyethylene terephthalate glycol (PeT-G), polypropylene (PP), polycarbonate (PC), thermoplastic polyurethanes (TPUs), and ethylene-vinyl acetate (EVA). The article concluded that all materials demonstrated good biocompatibility, with PeT-G and EVA aligners associated with relatively less tissue irritation. It should be emphasized, however, that since different studies employed varying cell models, lower irritation in some reports does not necessarily imply complete safety of the materials under all conditions.[7]

The purpose of the present study was to investigate the effects of materials used in myofunctional appliances on the viability of human periodontal ligament fibroblasts under simulated oral conditions. Additionally, it aimed to analyze the inflammatory responses and bone differentiation reactions of these cells.

Materials and methods

Preparation of artificial saliva (AS)

Artificial saliva (AS) was prepared by dissolving 0.8 mg of $CaCl_2 \cdot 2H_2O$, 0.4 mg of NaCl, 0.4 mg of KCl, and 1 mg of urea in 1000 ml of double-distilled water (ddH_2O) as described in reference.[8] The pH of the solution was adjusted to 6.7 using 1 N NaOH and 1 N HCl with a pH meter.[9] The solution was then transferred into serum bottles and sterilized in an autoclave at 121 °C and 15 psi for 20 min. After sterilization, the artificial saliva was cooled to room temperature and stored at 4 °C in a refrigerator for future use. The prepared artificial saliva was mixed with DMEM at ratios of 10 %, 20 %, 30 %, 40 %, 50 %, 75 %, and 100 %, respectively. The mixtures were transferred into test tubes and stored for subsequent use.

Preparation of test material extracts

Test materials, Preortho (PO, BioMaterials Korea Inc. Busan, Korea), EF line (EF, Orthoplus Co., Paris, France),

Table 1 The appliance information used in the experiment.

Products	Composition	Company
Preortho (Type1-MS)	Polyurethane	BioMaterials Korea Inc. Busan, Korea.
Myobrace (MRC) MYOBRACE® T1	Medical silicon, Polyurethane	Myofunctional Research Co., Helensvale, QLD, Australia.
Orthoplus (EF line)	Polyvinyl chloride, PVC	Orthoplus Co., Paris, France.
Invisalign (IV)	Thermoplastic Urethane (TPU)	Align Technology, Inc. San Jose, CA, USA

Myobrace (MB, Myofunctional Research Co., Helensvale, QLD, Australia.) and Invisalign (Align Technology, Inc. San Jose, CA, USA), were cut into cubic blocks of 0.5 cm^3 and sterilized with 75 % ethanol(Table 1). The blocks were air-dried in a laminar flow cabinet and then processed according to the ISO 10993-12 standard.[10] The materials were soaked in DMEM or DMEM containing artificial saliva at a concentration of 0.2 g/ml. The soaking process was conducted at 37 °C for 72 h. After soaking, the solutions were filtered through syringe filters and stored for further experiments.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay for cell viability

The MTT assay (Sigma Aldrich Co., St. Louis, MO, USA) was conducted to evaluate cell viability. HPDL cells were seeded into 24-well flat-bottomed tissue culture plates. After 24 h of incubation, the culture medium was replaced with 200 μL/well of the extract. After another 24-h incubation, the medium was replaced with 100 μL/well of MTT solution (1 mg/ml in PBS). The cells were incubated at 37 °C in a 5 % CO_2 atmosphere for 1 h. After incubation, the solution was removed, and 100 μL/well of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. The plates were gently swirled for 10 min, and optical density (OD) was measured at 540 nm using a spectrophotometer (Sunrise Co., Männedorf, Zurich, Switzerland).

Cell viability (%) was calculated using the formula:

$$\text{Cell Viability} = \frac{\text{OD of test group}}{\text{OD of control group}} \times 100$$

A survival rate above 70 % was considered safe for cell viability.

Detection in artificial saliva with different concentrations

HPLFs were evenly seeded into 24-well culture plates at a density of 2×10^4 cells per well and incubated at 37 °C with 5 % CO_2 for 24 h. After removing the old medium, fresh culture media mixed with different concentrations of artificial saliva were added to the wells. The plates were incubated for 24, 48, and 72 h under the same conditions. At each time point, the test material extracts were removed, and the cells were washed twice with PBS. MTT solution (1 mg/ml) was added, and the plates were incubated for 4 h at 37 °C with 5 % CO_2. After incubation, the MTT solution was removed, 600 μl of DMSO was added to dissolve the formazan crystals, and the solution was mixed thoroughly. A 150 μl aliquot from each well was transferred to a 96-well plate and analyzed using a microplate spectrophotometer at a wavelength of 570 nm. The experimental results were recorded and saved.

Detection with and without artificial saliva

HPLFs were evenly seeded into 24-well culture plates at a density of 2×10^4 cells per well and incubated at 37 °C with 5 % CO_2 for 24 h. After removing the old medium, test material extracts were added to the wells. The plates were incubated for 24, 48, and 72 h under the same conditions. At each time point, cell morphology was observed and recorded using an inverted microscope. The test material extracts were then removed, and the cells were washed twice with PBS. MTT solution (1 mg/ml) was added, and the plates were incubated for 4 h at 37 °C with 5 % CO_2. After incubation, the MTT solution was removed, 600 μl of DMSO was added to dissolve the formazan crystals, and the solution was mixed thoroughly. A 150 μl aliquot from each well was transferred to a 96-well plate and analyzed using a microplate spectrophotometer at a wavelength of 570 nm. The experimental results were recorded and saved.

Western blotting

Western Blotting was conducted to separate and detect specific proteins using SDS-PAGE gel electrophoresis. Proteins were separated based on their molecular weight, with smaller proteins migrating faster through the gel. The separated proteins were transferred onto a membrane, followed by specific antibody binding to detect the target protein levels.[11]

HPLFs (1.5×10^6 cells/dish) were seeded in 10 cm culture dishes and incubated at 37 °C with 5 % CO_2 for 24 h. After exposure to test material extracts for 24, 48, and 72 h, the medium was collected. Cells were washed with PBS, treated with 0.05 % trypsin, and centrifuged at 2000 rpm for 5 min. Cell pellets were washed with ice-cold PBS, lysed with RIPA buffer, and centrifuged at 10,000 rpm for 30 min at 4 °C. Proteins were quantified using the Bradford assay, mixed with loading dye, heat-treated, and stored at −80 °C. Protein samples (20 μg) were loaded onto SDS-PAGE gels and electrophoresed. Gels were run at 60 V (stacking gel) and 90 V (separating gel), then transferred to PVDF membranes using a transfer buffer at 100 V for 70 min. Membranes were blocked with blocking buffer for 1 h, incubated with primary antibody overnight at 4 °C, and washed with TBST. Secondary antibody was applied for 1 h, followed by TBST washes. Membranes were incubated with chemiluminescent substrate and analyzed using a chemiluminescence imaging system (LAS-4000, Fuji Photo Film Co., Ltd., Japan). Results were recorded for analysis.

Statistical analysis

Experimental data were recorded using Microsoft Office Excel 365 and quantitative analysis software (Science Lab 2005, Fuji Photo Film Co., Ltd., Tokyo, Japan). Statistical analysis was performed using JMP11 software (SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to evaluate differences between groups, with statistical significance set at $P < 0.05$. Post hoc comparisons were conducted using the Tukey–Kramer HSD test to identify specific group differences.

Results

A concentration of 30 % AS was found to have the least impact on HPLFs cell viability and was selected for subsequent experiments (Fig. 1). In the presence of AS, the EF eluate significantly reduced cell viability, particularly at 48 and 72 h, while Invisalign (IV) and Myobrace (MB) exhibited higher cell viability and lower cytotoxicity. Morphological observations revealed that EF Line caused near-complete structural damage to HPLFs with overall cell viability (Figs. 2 and 3).

The expression of inflammatory proteins (COX-2, IL-1, IL-6, TNF-α, and ERK) in HPLFs under different material eluates is shown in Figs. 4 and 5. The EF group exhibited significantly elevated inflammatory protein expression under both artificial saliva (AS) and non-AS conditions, with IL-6 and COX-2 peaking at 48 and 72 h. The PO group showed moderate inflammatory responses, with IL-1 and TNF-α significantly upregulated under AS conditions but remaining lower than EF. IV and MB groups displayed protein expression levels comparable to the control group, with minimal impact on cellular inflammatory responses ($P > 0.05$). The EF group demonstrated the highest inflammatory protein levels across all markers, indicating its strong pro-inflammatory effects, while IV and MB had the least impact.

The expression of bone remodeling-related proteins (ALP, OPG, and RANKL) under various material extracts is shown in Figs. 6 and 7. The control group displayed stable protein expression, serving as the baseline reference. EF exhibited the lowest ALP and OPG expression, with significantly elevated RANKL expression at 24 and 48 h, indicating a strong bone resorption effect. PO showed moderate RANKL expression and a slight increase in OPG at later stages. IV and MB demonstrated ALP and OPG expression close to the control, with low RANKL levels stabilizing after 48 h, suggesting minimal impact on bone remodeling. EF showed the highest RANKL/OPG ratio, emphasizing its bone resorption potential, while IV and MB maintained a favorable balance.

Discussion

The selection of materials for medical devices is a critical and evolving field, with ongoing research aimed at overcoming challenges related to biocompatibility, degradation control, and mechanical properties. Certain materials or their degradation products may exhibit cytotoxicity, causing harm or death to surrounding cells. This cytotoxicity can result in device failure, tissue damage, or systemic toxicity, posing significant health risks to patients.[12]

According to ISO 10993-12, for regular-shaped solid materials, the extraction ratio should be based on surface area per volume (e.g., cm^2/ml). In our study, the materials were soaked at a concentration of 0.2 g/ml, which we acknowledge corresponds to a **mass-to-volume ratio** rather than the **surface area-to-volume ratio (cm^2/ml)** recommended for regular-shaped solid materials under ISO 10993-12. Due to the irregular shapes of some material fragments after sectioning, which made accurate surface area

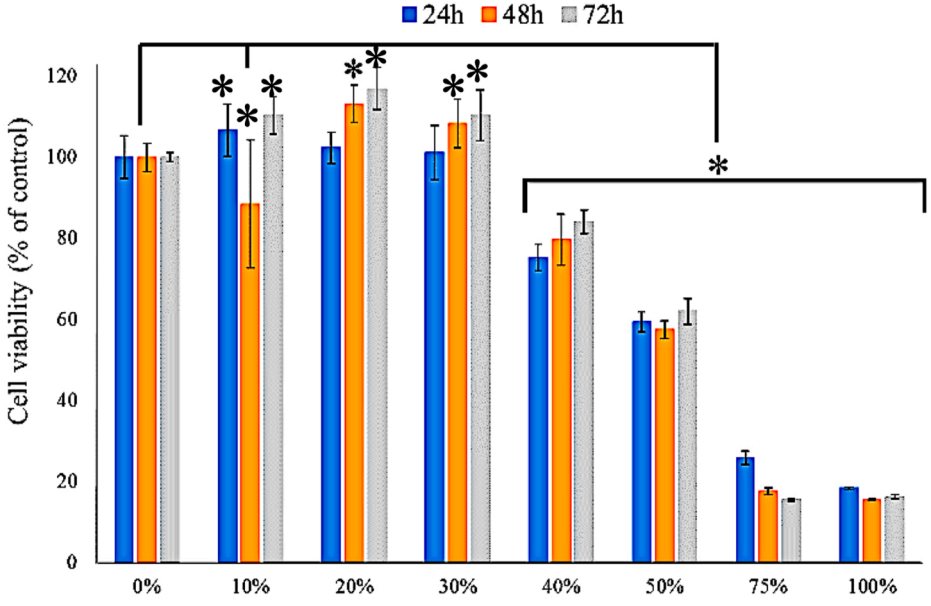

Figure 1 The comparison of the cell viability of HPLFs under different concentrations of artificial saliva. *$P < 0.05$.

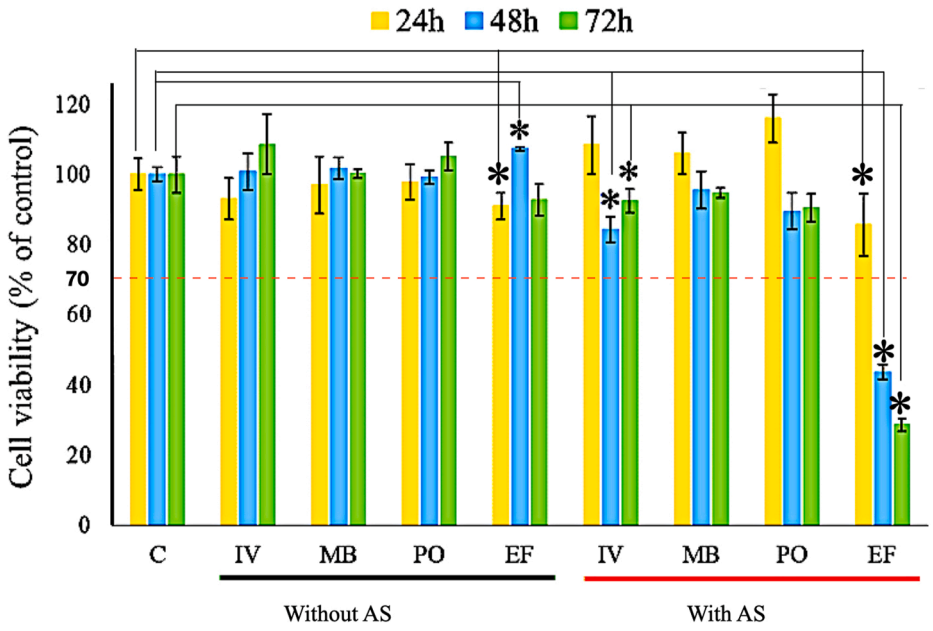

Figure 2 The effect of eluates from different devices, with or without saliva immersion, on the viability of periodontal ligament cells.

measurement difficult. Nevertheless, we standardized the preparation across all groups to ensure consistency within our experimental framework.

In the present study, we observed that a 30 % concentration of artificial saliva (AS) had the least impact on HPLF viability, whereas both higher and lower concentrations showed more pronounced effects on cell survival (Fig. 1). This finding suggests that the concentration of AS plays a critical role in maintaining cellular homeostasis. Although AS is designed to mimic the oral environment, its ionic composition and osmolarity differ from those of conventional culture media. At higher concentrations, AS may disrupt the osmotic pressure and ionic balance of the culture medium, leading to cellular stress and reduced viability. Conversely, at lower concentrations, the nutrient composition may become insufficient to support normal cell metabolism. Previous studies have similarly reported that the dilution of artificial saliva significantly influences cytotoxicity outcomes. The cytotoxic effects of dental materials varied depending on the concentration of AS used in vitro, highlighting the importance of dilution for preserving cell viability.[9] The ionic composition and osmolarity of saliva substitutes affected fibroblast proliferation and morphology, further supporting our findings.[13] Based on these observations, we suggest that a 30 % AS dilution provides a more physiologically balanced environment, minimizing osmotic stress while still maintaining relevant oral-like conditions. The present study, a 30 % concentration of AS had minimal impact on HPLFs cell viability and was used for further experiments. Without AS, eluates from the tested devices did not significantly affect cell viability. However, with AS, the EF Line eluate significantly reduced cell viability, especially at 48 and 72 h. Morphological analysis revealed severe damage to cell structure with EF Line, while IV and MB showed higher viability and lower cytotoxicity. Overall, cell viability ranked as: Control > Aligner ≈ Myobrace > ProOrtho > EF Line.

Due to the release of different substances from materials under various conditions, particularly in acidic and alkaline environments, one study found that the biological environment created by MB and LM Trainer (LM-Dental™, Parainen, Finland), with pH values below 0.9, does not exert toxic effects at the vascular level.[6] The elastodontic orthodontic appliances demonstrate adequate stability in biological environments due to the materials used in their construction, providing them with a biological profile suitable for clinical application.[6]

Although our results indicated that EF Line and PO eluates induced higher inflammatory responses compared to MB and IV, the underlying mechanisms remain to be fully clarified. One possible explanation could be related to changes in the pH of the culture medium, as previous studies have shown that acidic or alkaline conditions can compromise cell viability and stimulate inflammatory signaling. One study observed reduced cell viability of gingival fibroblasts exposed to elastodontic devices under acidic conditions.[6] However, it is important to note that in the present study we did not directly measure the pH of the material eluates. Therefore, the current data do not allow us to attribute the observed cytotoxicity of EF and PO to pH alterations with certainty. Instead, this explanation should be regarded as a plausible hypothesis that warrants further investigation. Future studies will need to include direct pH measurements of the eluates, in accordance with ISO 10993-5 guidelines, to determine whether pH shifts play a significant role in mediating the biological effects of elastodontic materials.

TPU is valued for its flexibility, transparency, and resistance to oil. As it typically does not contain BPA, it does not release bisphenol A (BPA) under normal conditions. TPU is widely used in applications requiring a combination of flexibility and durability. In the medical field, TPU has demonstrated effectiveness beyond bone and cartilage regeneration. Studies on a TPU and polylactic acid (PLA)

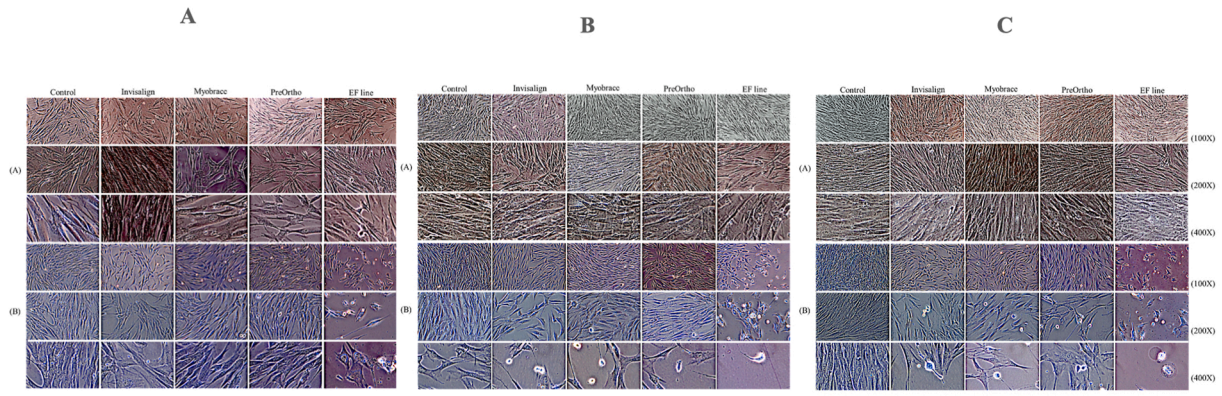

Figure 3 Morphological changes of HPLFs after various hours exposure to different material eluates.
Group A: Cell morphology under conditions without artificial saliva.
Group B: Cell morphology under conditions with artificial saliva
A. In the control group, cells maintained an intact morphology, exhibiting a spindle-shaped distribution with tightly packed and well-organized alignment.
In the Invisalign group, Group A: Cell morphology remained largely normal, with slight sparsity observed in some areas. Group B: Cell morphology was altered compared to the control group, with some cells exhibiting shrinkage or deformation. In the Myobrace group, Group A: Cells maintained a good morphology, with density comparable to the control group. Group B: Cells appeared slightly sparse, but their morphology remained intact, though intercellular gaps increased. In the ProOrtho group, Group A: Cell arrangement appeared slightly disorganized but remained largely intact. Group B: More pronounced morphological changes were observed, with signs of cell degradation and decreased cell density. In the EF Line group, Group A: Cells exhibited irregular morphology, with some showing shrinkage or cell death. Group B: More severe damage was observed, with a large number of cells showing shrinkage or signs of detachment.
B. Morphological changes of HPLFs after 48-h exposure to different material eluates (Fi. 3-2). In the control group (A/B), cells exhibited regular alignment, intact morphology, a healthy spindle-shaped structure, and high density. In the Invisalign group, Group A: Cell morphology was similar to the control group, with intact structure and slightly looser arrangement. Group B: Some cell deformation was observed, but cell viability remained relatively high, with slightly increased intercellular spacing. In the Myobrace group, Group A: Cells maintained an intact morphology, with a slight reduction in density compared to the control group. Group B: Intercellular spacing increased, and some cells exhibited disorganized alignment. In the ProOrtho group, Group A: Cell density was significantly reduced, with some cells showing shrinkage. Group B: More pronounced cellular damage was observed, with abnormal morphology and signs of cell degeneration or death. In the EF Line group, Group A: Cells exhibited disorganized alignment, low density, and most cells showed shrinkage or signs of cell death. Group B: Cellular damage was more severe, with nearly no observable normal cells.
C. Morphological changes of HPLFs after 72-h exposure to different material eluates. In the control group, Group A & Group B: Cells exhibited a regular spindle-shaped arrangement, uniform distribution, and high density, indicating good cell health. In the Invisalign Group, Group A: Cells appeared slightly dispersed but maintained an intact spindle-shaped structure. Cell spacing was slightly increased, with mildly irregular alignment. Group B: Cells began to show deformation, and cell density was reduced. In the Myobrace Group, Group A: Cell morphology was similar to the control group, though slightly disorganized, with a slight reduction in density. Group B: Cellular damage increased slightly, with some cells exhibiting shrinkage and irregular morphology. In the ProOrtho Group, Group A: Cells displayed significant disorganization, reduced density, and noticeable shrinkage in some areas. Group B: Cellular morphology further deteriorated, with an increased number of dead cells and significantly larger intercellular spaces. In the EF Line Group, Group A: Cells exhibited highly abnormal morphology, disorganized structure, and extremely low density. A large number of cells showed shrinkage or signs of cell death. Group B: Cellular damage was severe, with nearly all cells exhibiting abnormal morphology and widespread cell death.

composite have revealed that the TPU-to-PLA ratio significantly influences the proliferation, metabolism, adhesion, growth, and interactions of human adipose-derived mesenchymal stromal stem cells.[14] These findings highlight TPU's excellent biocompatibility and BPA-free nature.

Medical devices, including orthodontic training appliances and oral protective devices, commonly use materials such as polyvinyl acetate polyethylene copolymer and polyvinyl chloride (PVC).[15] PVC's impact on cytokine release is influenced by particle density, morphology, and surface chemistry. One study showed no endotoxins in PVC particles, but their physical toxicity, driven by high density and irregular shapes, may slightly promote cytokine release.[16]

EF was identified as the material inducing the strongest inflammatory response, particularly with significant expression of IL-6 and COX-2, while PO exhibited a moderate level of inflammatory reaction (Fig. 5). In contrast, Invisalign and MB had minimal effects on inflammatory proteins, demonstrating good biocompatibility. The inflammatory effects of EF and PO accumulated over time, peaking between 48 and 72 h. EF and PO materials may not be suitable for inflammation-sensitive environments and require further modification of their chemical components

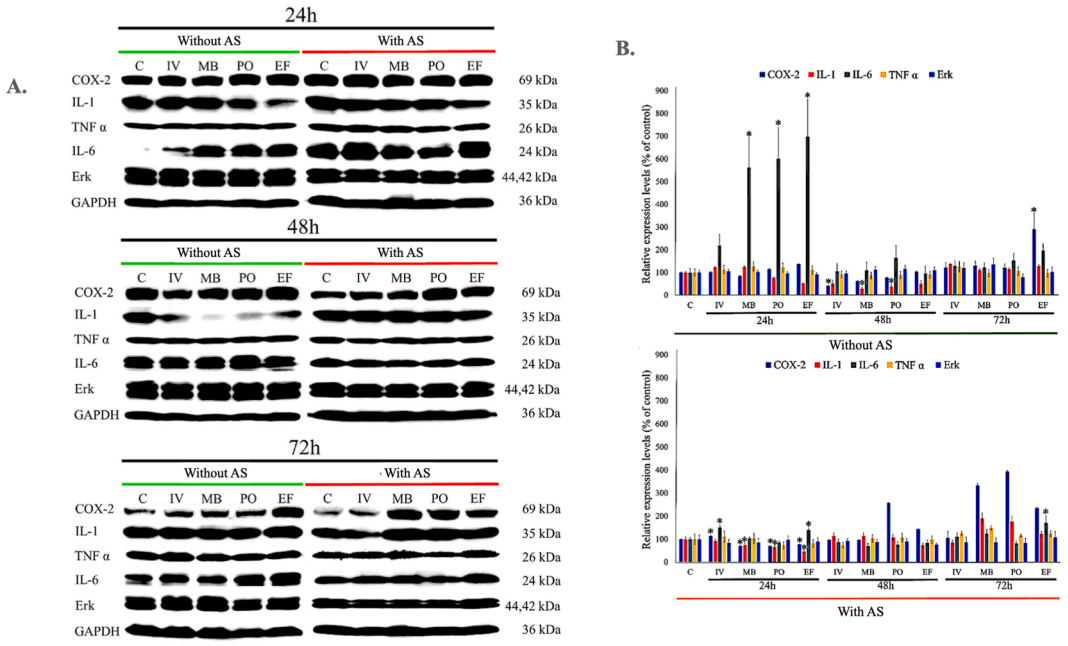

Figure 4 A. Expression of inflammatory proteins in HPLFs exposed to different material eluates. B. Bar chart showing the relative protein expression levels (%) compared with the control group.

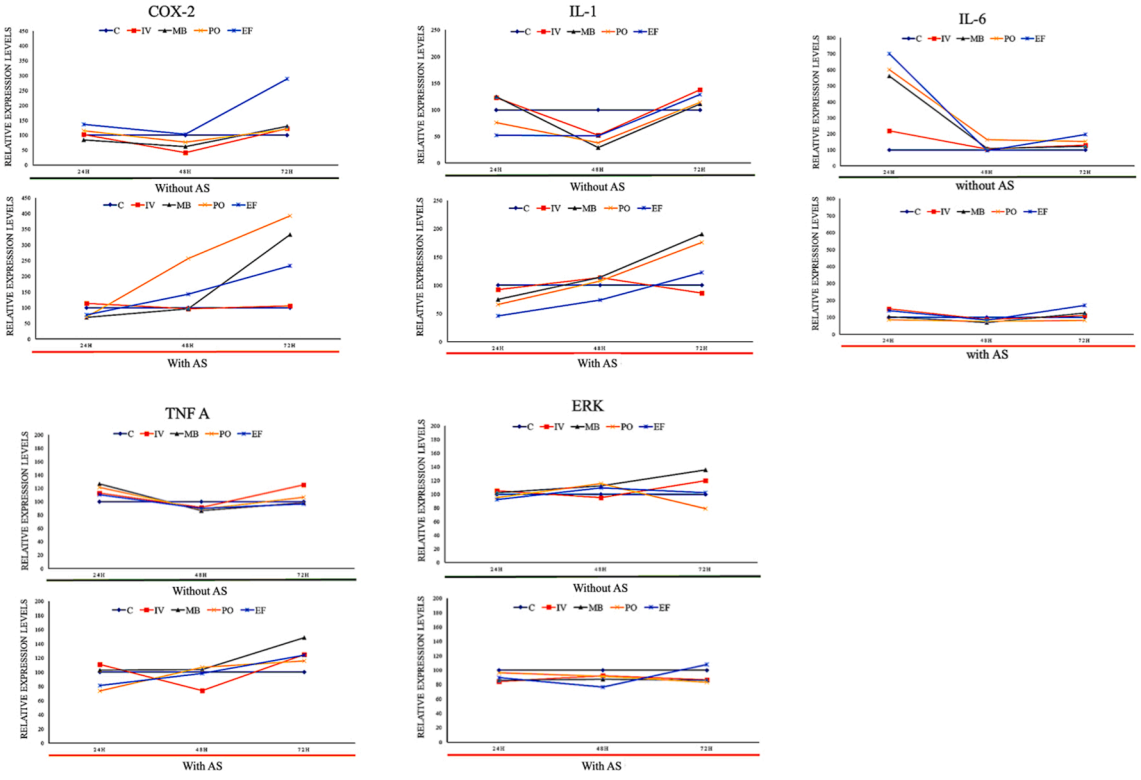

Figure 5 The line graph illustrates the dynamic trends of inflammatory protein expression across different time points for various materials.

to reduce cytotoxicity and inflammation induction. Conversely, IV and MB could be considered as candidates with higher biosafety.

In addition to the observed differences in cytotoxicity and inflammatory responses, it is important to consider the potential mechanisms underlying these findings based on

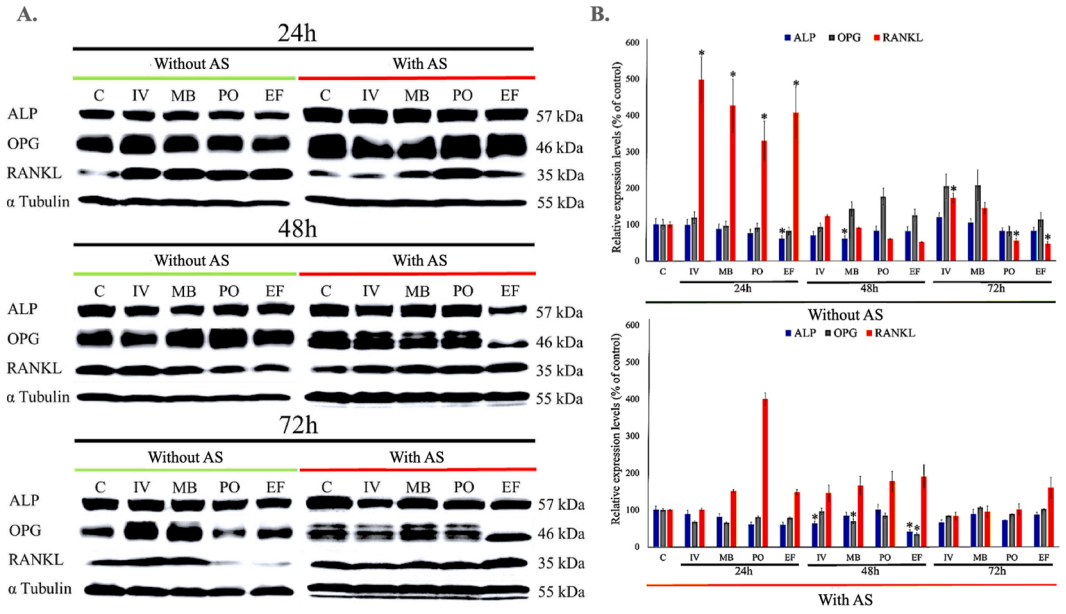

Figure 6 **A.** The expression of bone remodeling-related proteins, (Alkaline phosphatase (ALP), Osteoprotegerin (OPG), Receptor activator of nuclear factor Kappa-B ligand (RANKL) was observed at different time points (24 h, 48 h, and 72 h) under various material extracts and culture conditions. **B.** The bar graphs show the protein expression percentage of each material relative to the control at specific time points.

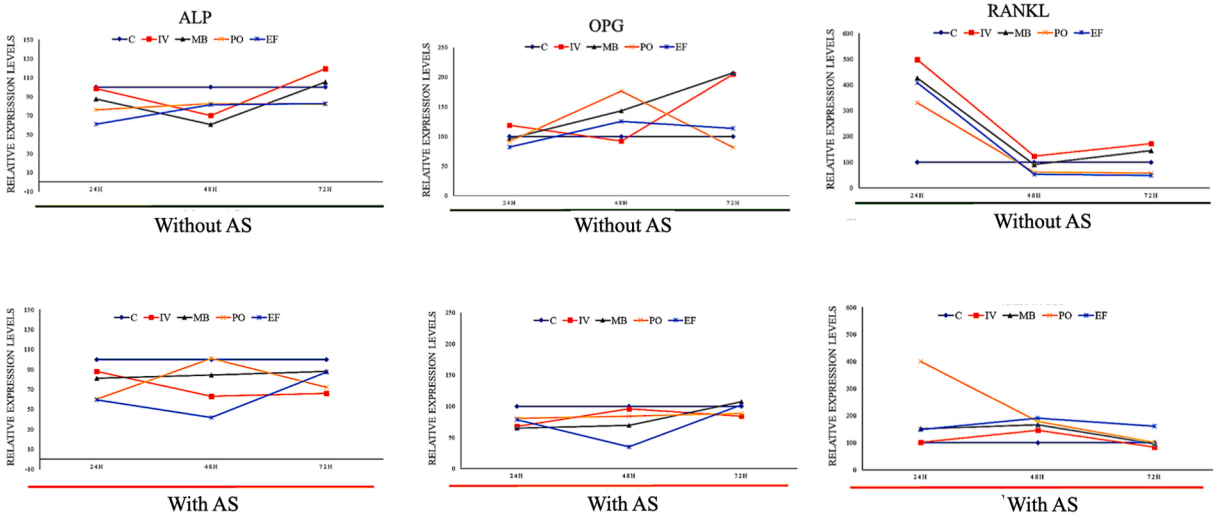

Figure 7 The curve graphs illustrate the temporal changes in HPLFs protein expression.

the chemical composition of the tested materials. The EF Line device is primarily composed of **polyvinyl chloride (PVC).** Although widely used, PVC may release additives such as **plasticizers (e.g., phthalates)** and other degradation products under certain conditions. These leachables have been reported to stimulate inflammatory pathways, including the upregulation of cytokines such as IL-6 and COX-2,[16] which may explain the elevated inflammatory markers in the EF group.

The PO appliance is based on **polyurethane (PU).** While PU is generally considered biocompatible, studies indicate that its degradation can yield **isocyanates or low—molecular weight oligomers,** which exert mild cytotoxic and pro-inflammatory effects in vitro.[12,17] This could account for the moderate inflammatory and bone metabolism—related changes observed in our experiments. In contrast, the Invisalign aligner, manufactured from **thermoplastic polyurethane (TPU),** and the Myobrace device, composed of **medical-grade silicone and PU,** showed minimal cytotoxicity. TPU is noteworthy in that it does not contain bisphenol A (BPA) and exhibits relatively stable degradation under physiological conditions, thereby reducing the likelihood of releasing toxic leachables. This may explain why these appliances induced lower levels of cytotoxic and inflammatory responses compared to EF and PO. Taken together, these observations suggest that the

cytotoxic and inflammatory effects are not intrinsic to the bulk polymers themselves but may arise from leachable degradation products or unreacted monomers associated with specific materials. Further studies focusing on the identification and quantification of such substances will be essential to fully elucidate the mechanisms of toxicity.

EF exhibited a strong promotion of bone resorption, particularly characterized by high RANKL expression and low OPG expression, while PO showed a moderate induction of bone resorption, with potential compensatory effects in later stages. IV and MB were the closest to the control group, demonstrating good balance in bone remodeling (Fig. 6). The effects of EF were most pronounced in the early phase (24—48 h), whereas PO exhibited a slight protective effect at 72 h. EF may not be suitable for bone-related applications and requires further investigation into its impact on bone remodeling. Although PO showed moderate effects, its early promotion of bone resorption warrants caution. IV and MB appear to be safer options with better biocompatibility for bone-related applications.

In conclusion, materials used in orthodontic devices demonstrated varying impacts on biocompatibility and cellular responses. MB and IV showed higher cell viability, minimal inflammatory effects, and stable bone remodeling properties, making them suitable for clinical applications. In contrast, EF Line and PO exhibited significant cytotoxicity, inflammatory responses, and a tendency to promote bone resorption, particularly under acidic or alkaline conditions.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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